

Figure 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

	HindIII	NcoI	2H7 V _L Leader Peptide→
1	AAGCTTGGCG CC	ATGGATTTCAGAG ATTTTCAGCT TCCGTCAAT CAGTGCTTC	M D F Q V Q I F S F L L I S A S
61	V I I A R G Q I V L S Q S P A I L S A S	GTCATAATTG CCAGAGGACA ATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT	2H7 V _L →
121	P G E K V T M T C R A S S S V S Y M H W	CCAGGGGAGA AGGTACAAAT GACTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG	
181	Y Q Q K P G S S P K P W I Y A P S N L A	TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTG ATGCCCATC CAACCTGGCT	BamHI
241	S G V P A R F S G S G S G T S Y S L T I	TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC	
301	S R V E A E D A A T Y Y C Q Q W S F N P	AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA	
361	P T F G A G T K L E I K G G G G S G G G	CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT	(Gly ₄ Ser) ₃ Linker
421	G S G G G G S S Q A Y L Q Q S G A E L V	GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG	2H7 V _H →
481	R P G A S V K M S C K A S G Y T F T S Y	AGGCCTGGGG CCTCAGTGAA CATGTCCTGC AAGGTTCTG GCTACACATT TACCAAGTTAC	
541	N M H W V K Q T P R Q G L E W I G A I Y	AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT	
601	P G N G D T S Y N Q K F K G K A T L T V	CCAGGAAATG GTGATACTTC CTACAATCAG AAGTCAAGG GCAAGGCCAC ACTGACTGTA	
661	D K S S S T A Y M Q L S S L T S E D S A	GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGG	
721	V Y F C A R V V Y Y S N S Y W Y F D V W	GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGCTGG	

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Figure 1 B

BclI

~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGGA CCACGGTCAC CGTCTCT**GAT** CAGGAGCCCA AATCTTGTA CAAACTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAACTA CAAGACACAG CCTCCCGTGC TGGACTCCGA CGGCTCCCTC

1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGAA CGTCTCTCA

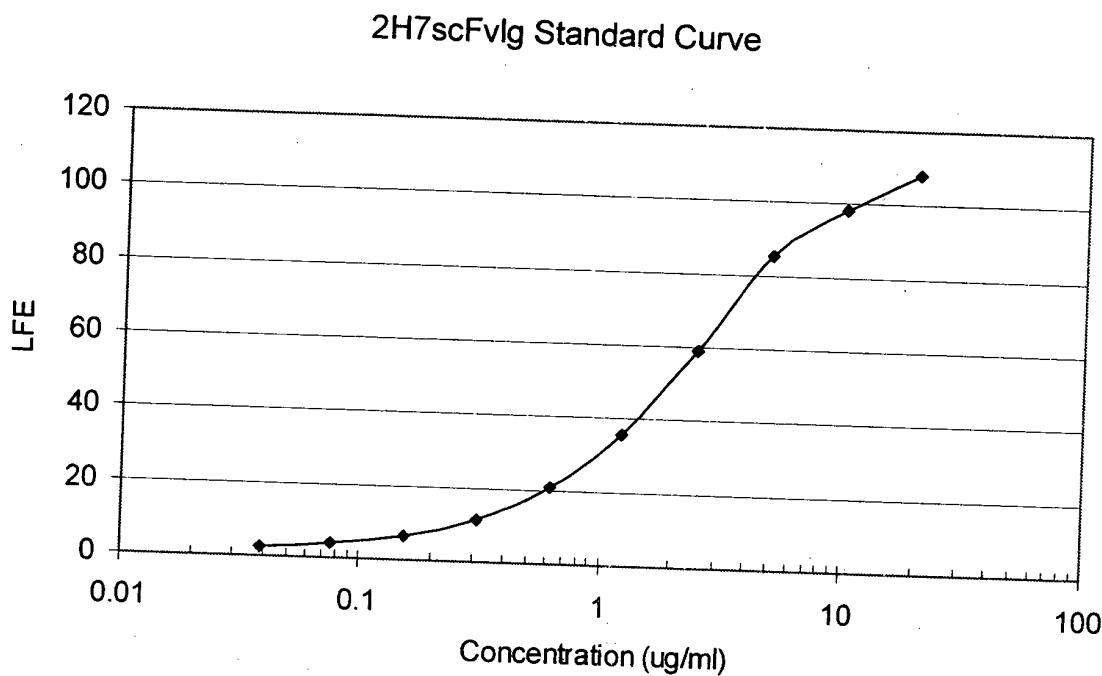
1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI

~~~~~

1501 P G K * S R
CCGGGTAAAT **GATCTAGA**

Figure 2.



Clone	LFE @ 1:50	Estimated Concentration (μg/ml)
D2	26.1	56
IIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

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Inventor: Jeffrey A. Ledbetter et al. Docket No. 3944-401
EXPRESS MAIL NO. EL 755733415US

Figure 3.

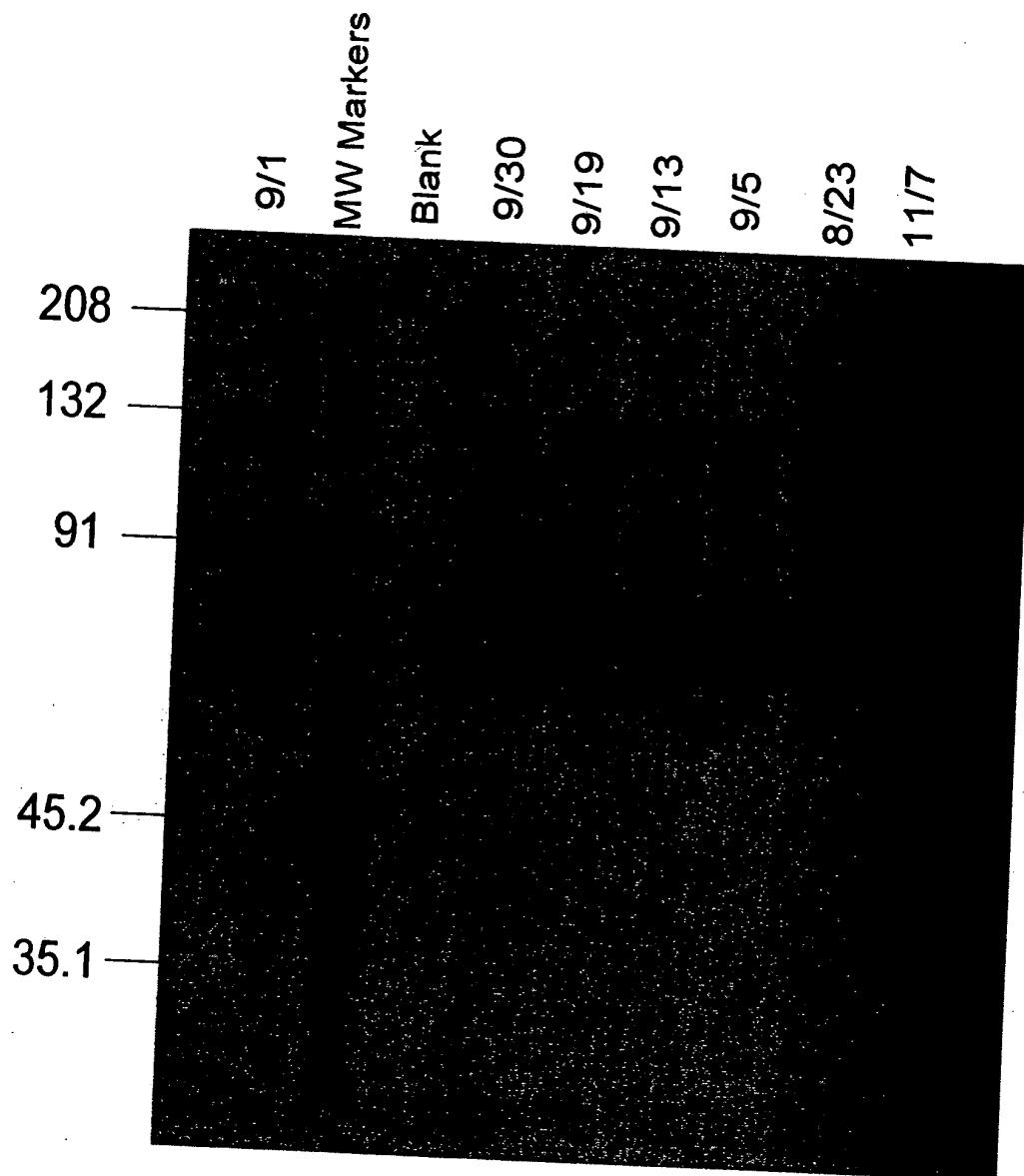


Figure 4A.

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:

2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Figure 4B.

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:

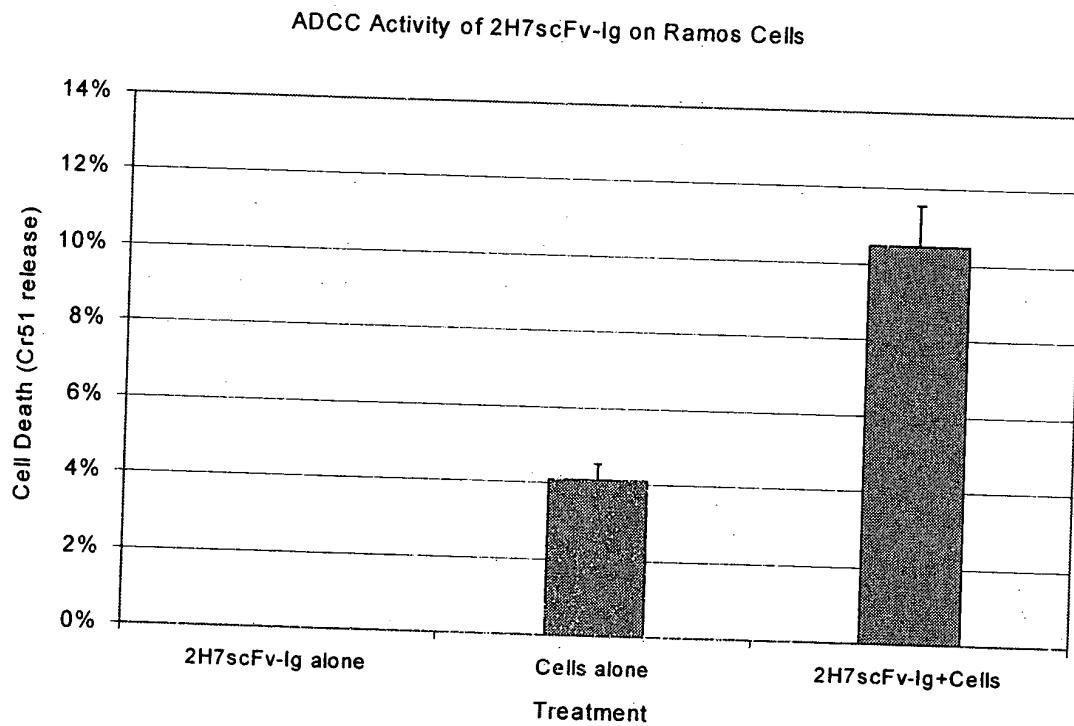


Figure 5.

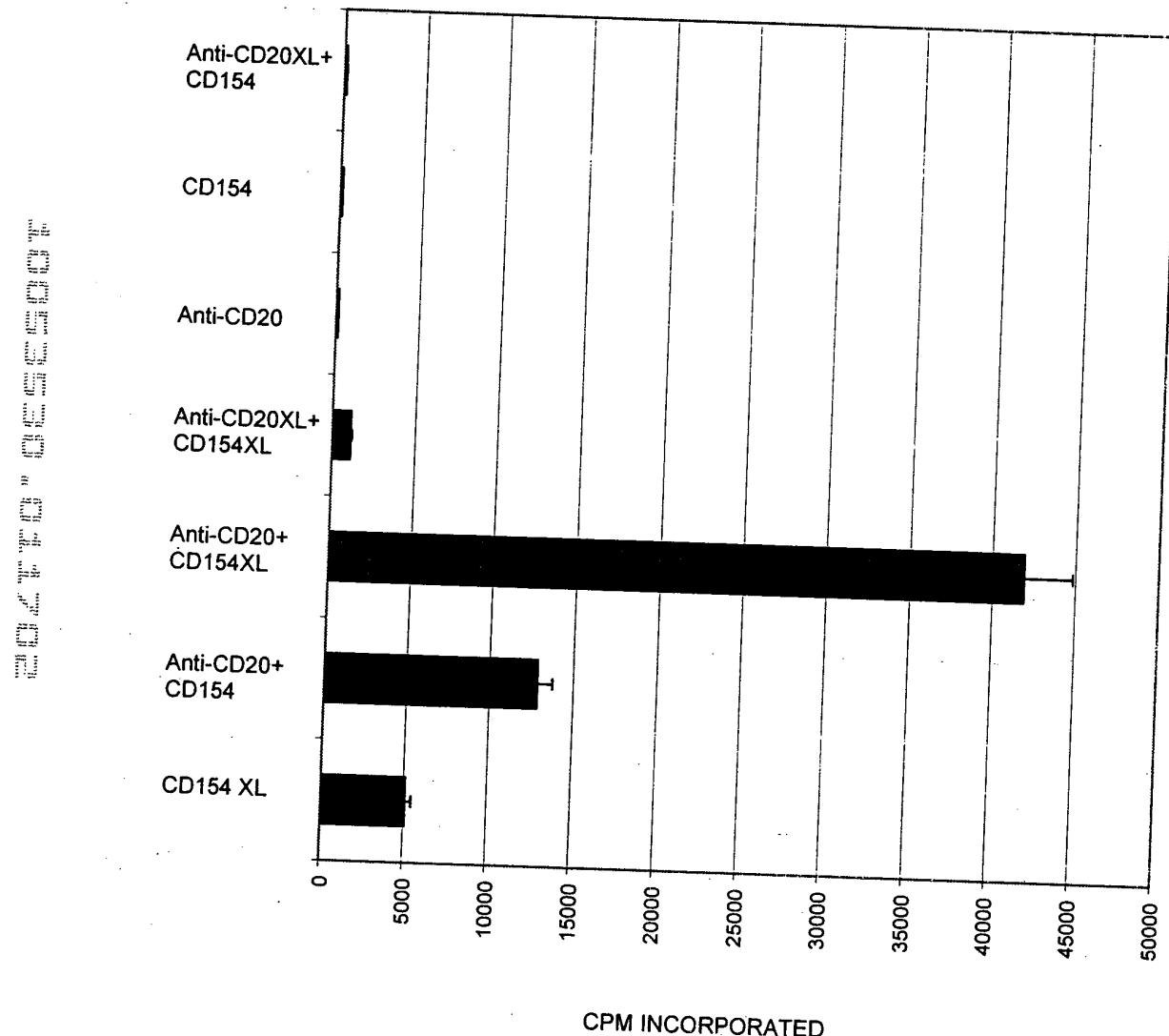
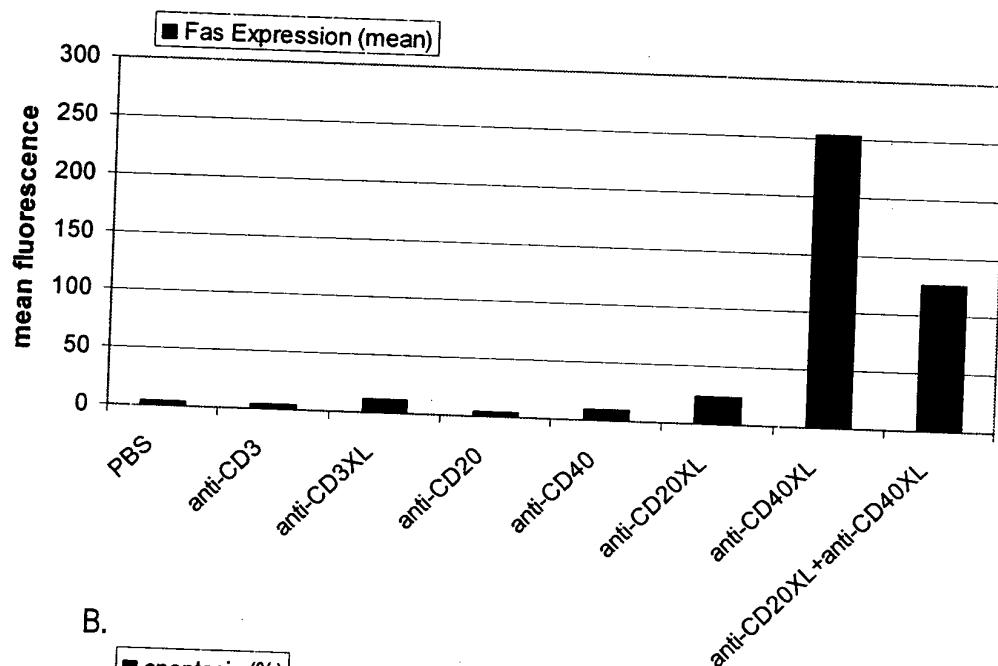


Figure 6A and B.

A.



B.

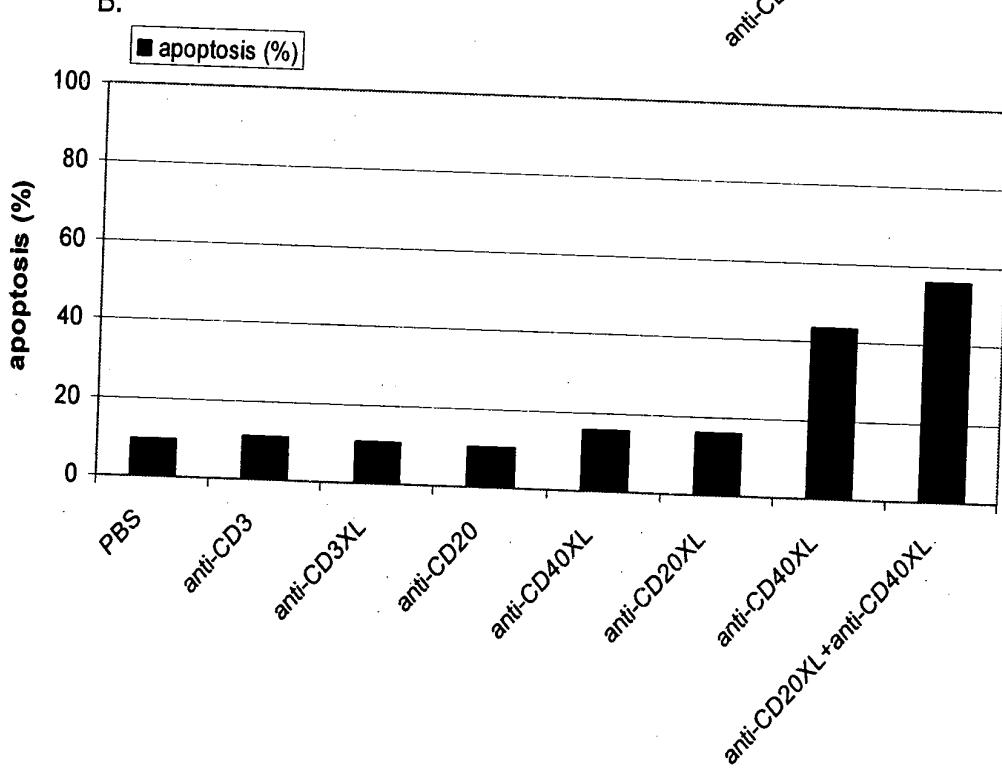


Figure 7A.

2H7-CD154 L2 cDNA and predicted amino acid sequence:

HindIII	NcoI 2H7 V _L Leader Peptide →
~~~~~	
1	AAGCTTGCCG CC M D F Q V Q I F S F L L I S A S
ATGGATTT TCAAGTGAG ATTTCAGCT TCCTGCTAA CAGTGCTTC	
2H7 V _L →	
61	V I I A R G Q I V L S Q S P A I L S A S
GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT	
121	P G E K V T M T C R A S S S S V S Y M H W
CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG	
BamHI	
~~~~~	
181	Y Q Q K P G S S P K P W I Y A P S N L A
TACCAGCAGA AGCCAGGATC CTCCCCAAA CCTGGATT ATGCCCATC CAACCTGGCT	
241	S G V P A R F S G S G S G T S Y S L T I
TCTGGAGTCC CTGCTCGTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC	
301	S R V E A E D A A T Y Y C Q Q W S F N P
AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA	
(Gly ₄ Ser) ₃ Linker →	
361	P T F G A G T K L E L K G G G G S G G G
CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT	
2H7 V _H →	
421	G S G G G G S S Q A Y L Q Q S G A E L V
GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG	
481	R P G A S V K M S C K A S G Y T F T S Y
AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTCTG GCTACACATT TACCAGTTAC	
541	N M H W V K Q T P R Q G L E W I G A I Y
AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCTGG AATGGATTGG AGCTATTAT	
601	P G N G D T S Y N Q K F K G K A T L T V
CCAGGAAATG GTGATACTTC CTACAATCAG AAGTCAAGG GCAAGGCCAC ACTGACTGTA	
661	D K S S S T A Y M Q L S S L T S E D S A
GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG	
721	V Y F C A R V V Y Y S N S Y W Y F D V W
GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACCTCTT ACTGGTACTT CGATGTCTGG	

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Figure 7B

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 G T G T T V T V S D P R R L D K I E D E
 GGCACAGGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E
 AGGAATCTTC ATGAAGATTG TGTATTGATG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K
 AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G
 GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAGGT

BclI

1021 D Q N P Q I A A H V I S E A S S K T T S
 GATCAGAACATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E
 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTGGT AACCTGGAA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T
 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

1201 F C S N R E A S S Q A P F I A S L C L K
 TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAT GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K
 TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1321 P C G Q Q S I H L G G V F E L Q P G A S
 CCTTGCAGGGC AACAAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

1381 V F V N V T D P S Q V S H G T G F T S F
 GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XbaI

1441 G L L K L E * * S R
 GGCTTACTCA AACTCGAGTG ATAATCTAGA

Figure 7C

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

HindIII NcoI
 ~~~~~~ ~~~~~~ **2H7 V<sub>L</sub> Leader Peptide →**  
 1    AAGCTTGC CG CC    ATGGATTT TCAAGTGCAG ATTTCAGCT TCCTGCTAAT CAGTGCTTCA  
 61    V I I A R G Q I V L S Q S P A I L S A S  
 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT  
 121    P G E K V T M T C R A S S S V S Y M H W  
 CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG  
 BamHI  
 ~~~~~~  
 181 Y Q Q K P G S S P K P W I Y A P S N L A
 TACCAAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT ATTGCCCATC CAACCTGGCT
 241 S G V P A R F S G S G S G T S Y S L T I
 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC
 301 S R V E A E D A A T Y Y C Q Q W S F N P
 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA
 361 P T F G A G T K L E L K G G G G S G G G (**Gly₄Ser₃**) Linker →
 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT
 421 G S G G G G S S Q A Y L Q Q S G A E L V
 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGT
 481 R P G A S V K M S C K A S G Y T F T S Y
 AGGCCTGGGG CCTCAGTGAA GATGTCTGC AAGGCTCTG GCTACACATT TACCAAGTTAC
 541 N M H W V K Q T P R Q G L E W I G A I Y
 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT
 601 P G N G D T S Y N Q K F K G K A T L T V
 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTCAAGG GCAAGGCCAC ACTGACTGTA
 661 D K S S S T A Y M Q L S S L T S E D S A
 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG
 721 V Y F C A R V V Y Y S N S Y W Y F D V W
 GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

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Inventor: Jeffrey A. Ledbetter et al. Docket No. 39-401
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Figure 7D.

human CD154/amino acid 108 →

G T G T T V T V S D P E N S F E M Q K G BclI/Bam hybrid site BclI
781 GGCACAGGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT
BclI
~~~~~  
D Q N P Q I A A H V I S E A S S K T T S      BclI  
841 GATCAGAACAT CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT  
V L Q W A E K G Y Y T M S N N L V T L E  
901 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTGGAA  
N G K Q L T V K R Q G L Y Y I Y A Q V T  
961 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC  
HindIII  
~~~~~  
F C S N R E A S S Q A P F I A S L C L K
1021 TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAA GCTCCATTAA TAGCCAGCCT CTGCCTAAAG
S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA
P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTTGCGGGC ACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG
NcoI
~~~~~  
V F V N V T D P S Q V S H G T G F T S F  
1201 GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCCTT  
XbaI      XbaI  
~~~~~  
G L L K L E * * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA

Figure 8.

CD20 CHO cell targets + (control or fusion protein)
+ Biotin-CD40Ig + PE-SA

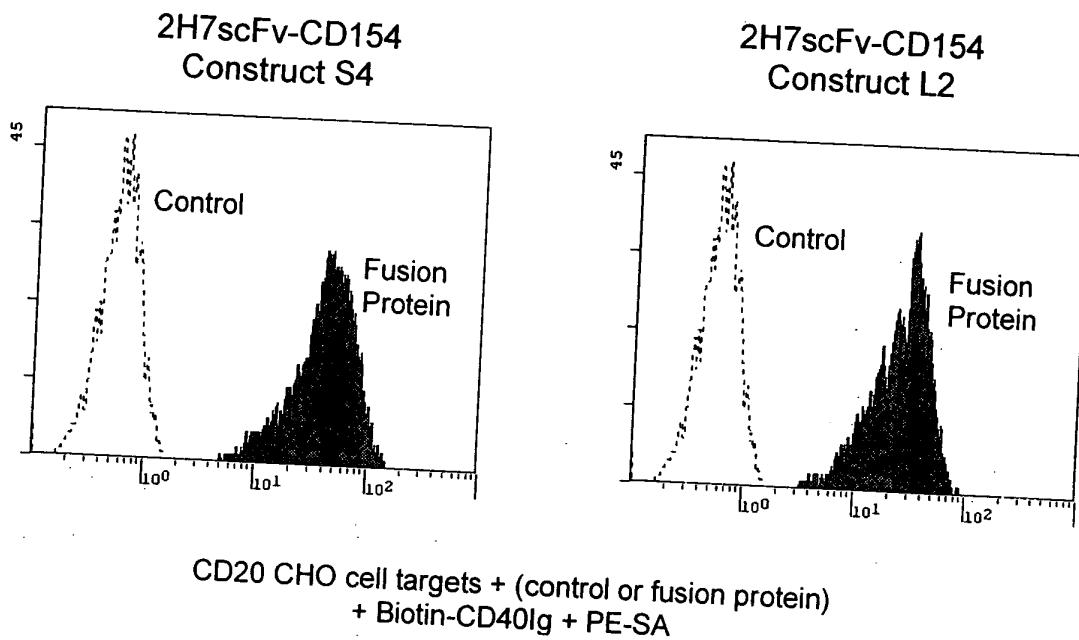
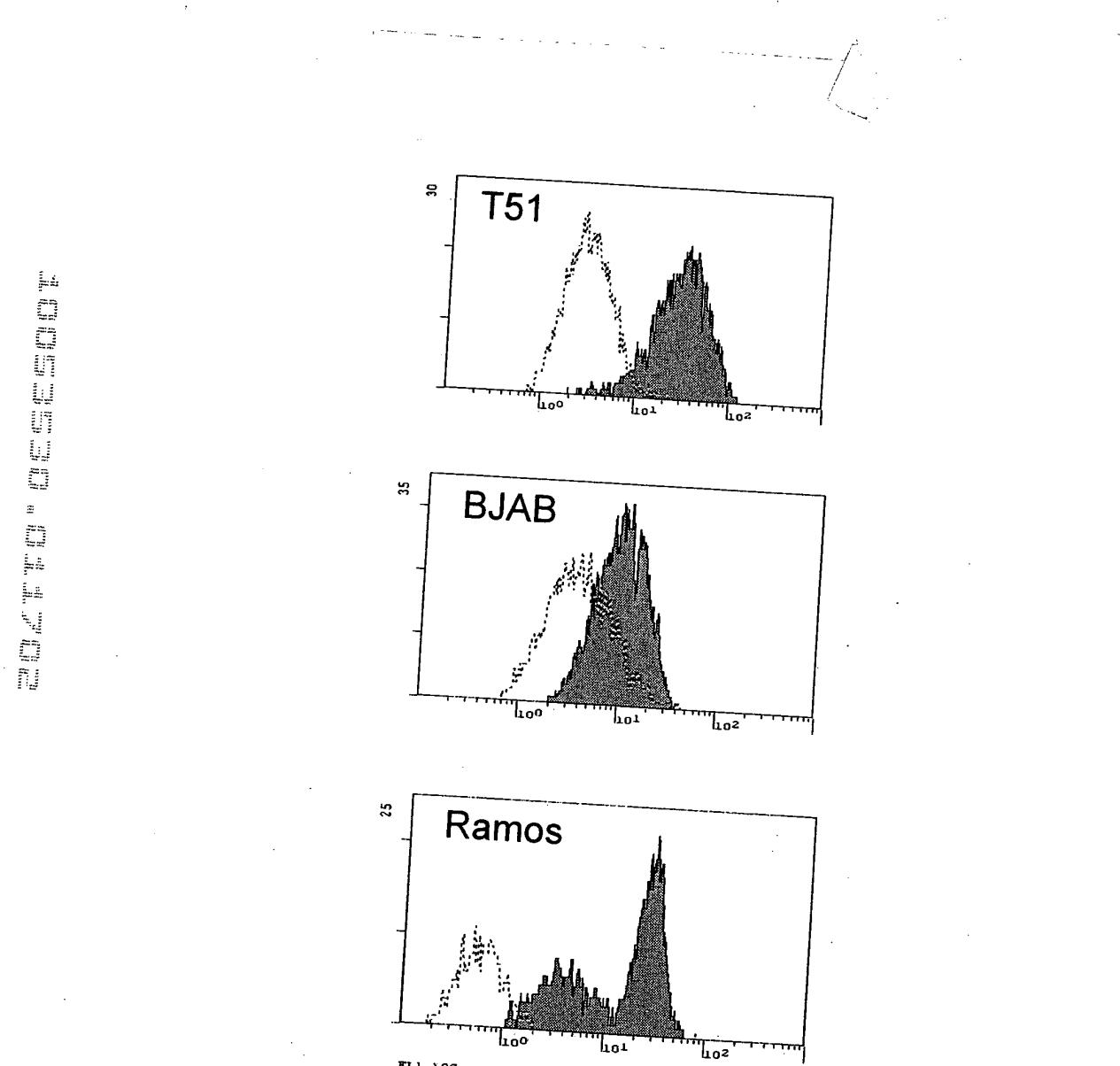
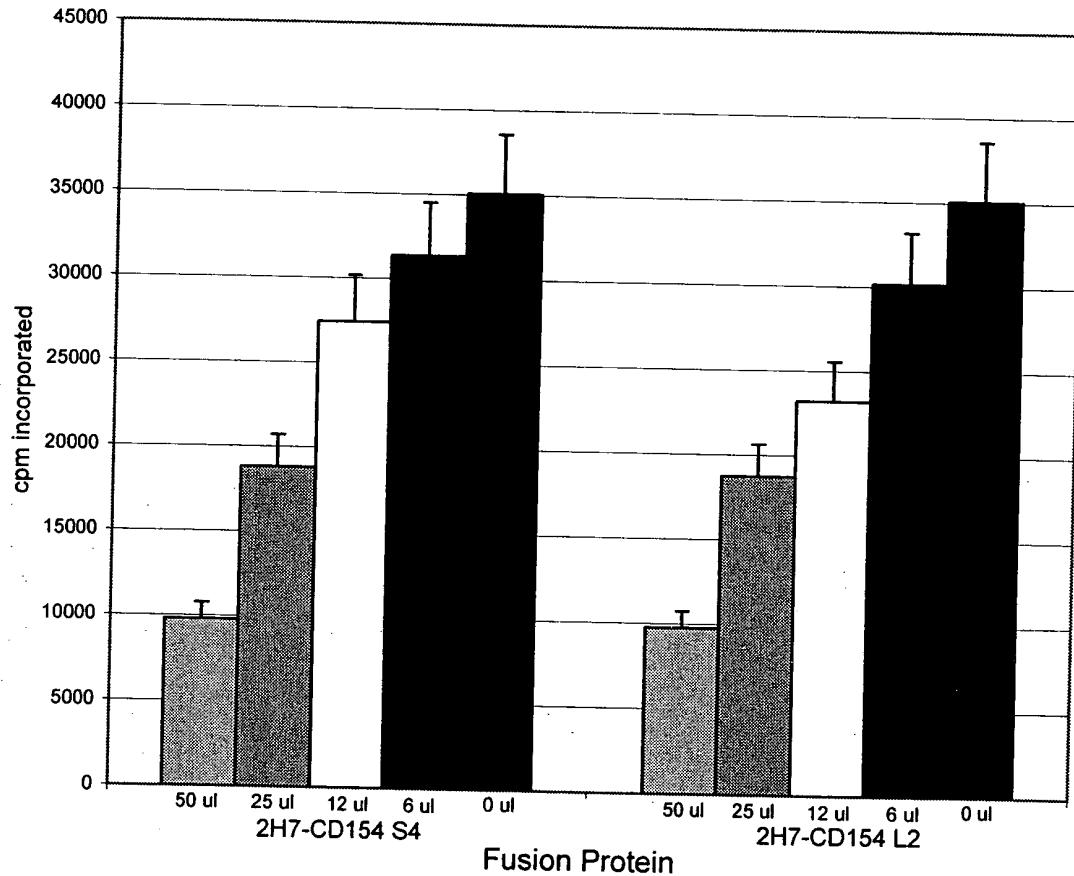


Figure 9.



.....control supernatant 2H7scFv-CD154 supernatant

Figure 10.



TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS

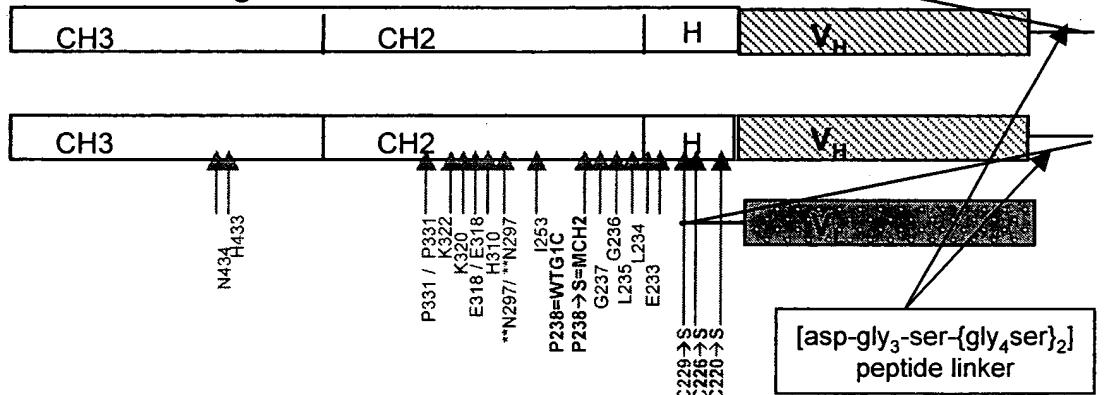
Inventor: Jeffrey A. Ledbetter et al. Docket No. 39-14401

EXPRESS MAIL NO. EL 755733415US

FIGURE 11

CytoxB-MHWTG1C
OR CytoxB-MHMG1C:

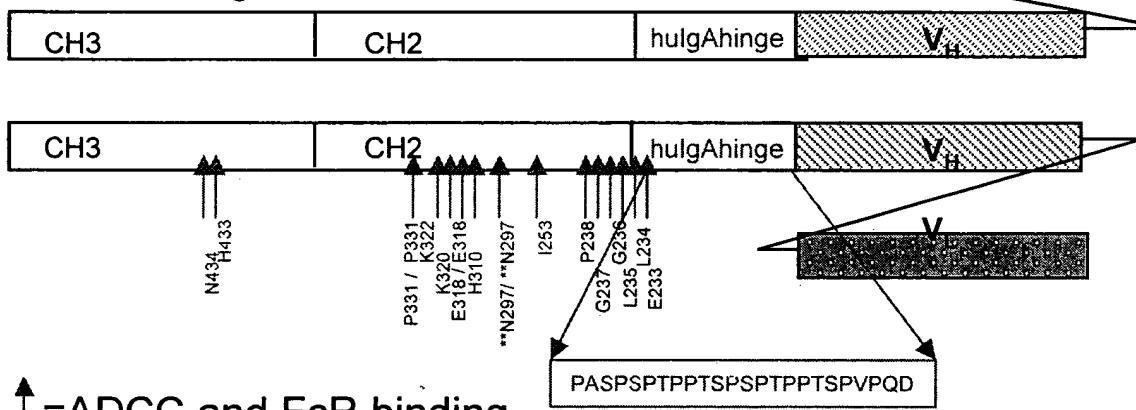
Human IgG1 CH2-CH3



2H7 scFv

CytoxB-IgAHWTHG1C:

Human IgG1 CH2-CH3



2H7 scFv

↑ =ADCC and FcR binding

↑ =Complement Fixation

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FIGURE 12

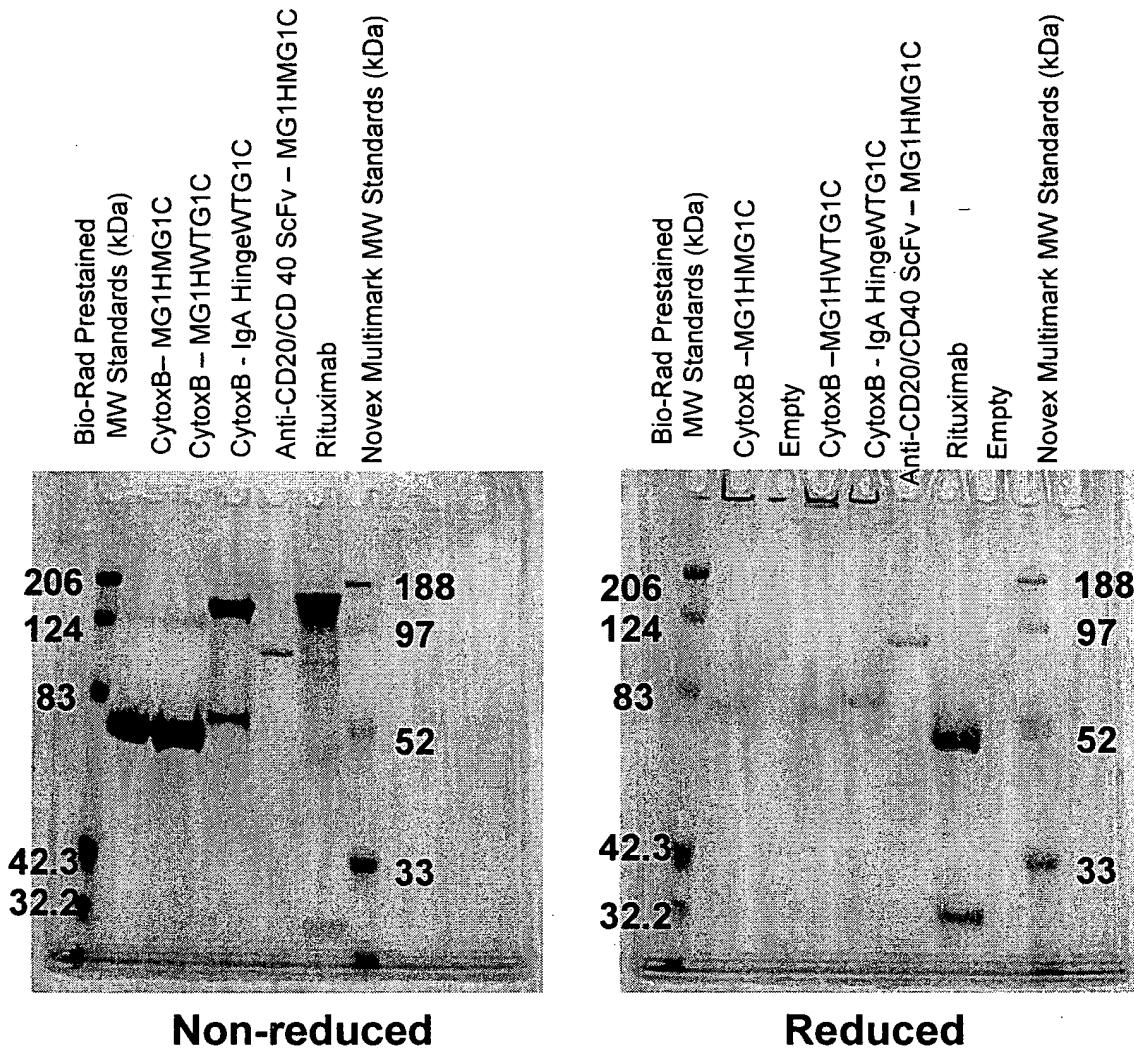
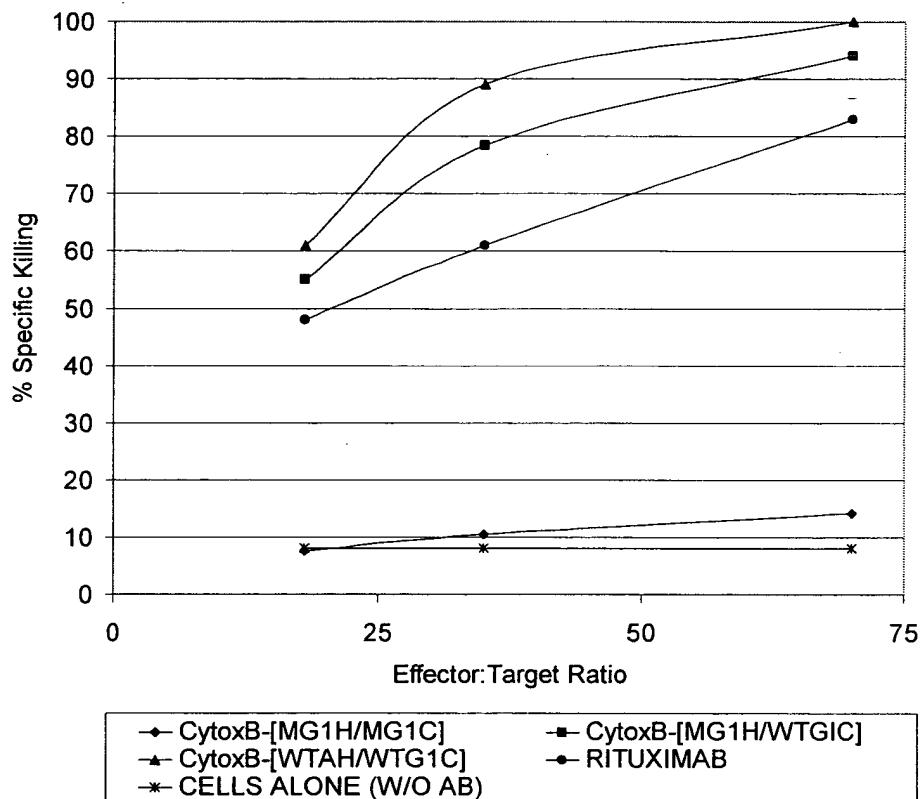
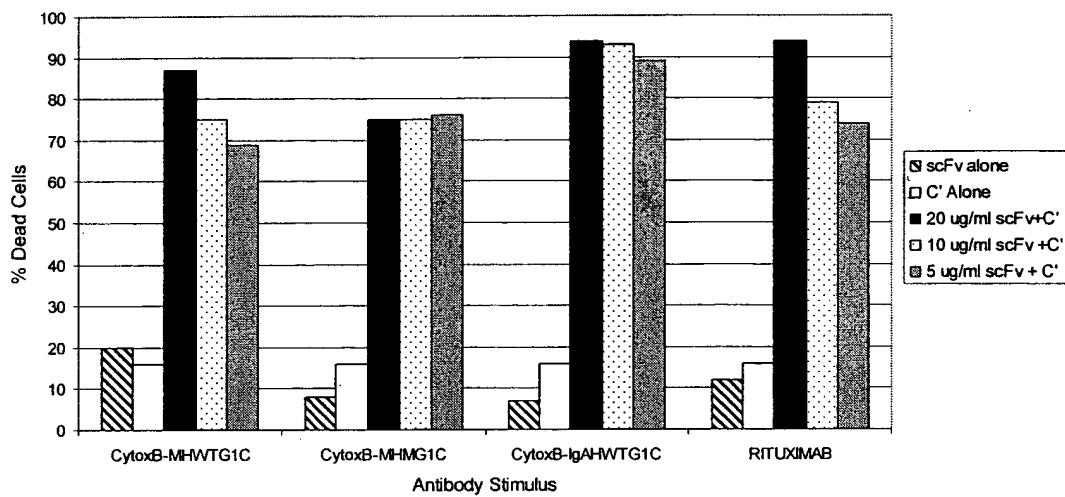


FIGURE 13



TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS
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FIGURE 14

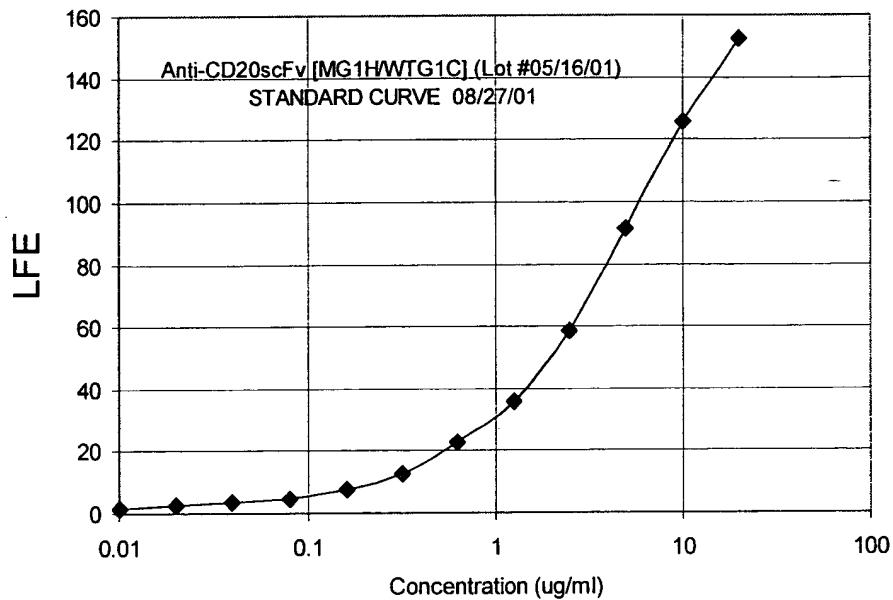


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Inventor: Jeffrey A. Ledbetter et al. Docket No. 3700-401

EXPRESS MAIL NO. EL 755733415US

FIGURE 15



Day	Monkey J99231		Monkey K99334	
	LFE(1:40)	Concentration (μ g/mL)	LFE(1:40)	Concentration (μ g/mL)
Injection → -7	2.41	<0.6 μ g/mL	1.51	<0.4 μ g/mL
0	2.22	<0.6 μ g/mL	1.63	<0.4 μ g/mL
Injection → 1	73.8	220 μ g/mL	44.4	100 μ g/mL
3	20.0	28 μ g/mL	40.2	80 μ g/mL
7	15.6	24 μ g/mL	15.7	24 μ g/mL
8	39.1	80 μ g/mL	42.6	92 μ g/mL
10	11.5	18 μ g/mL	2.74	1.2 μ g/mL
14	2.05	0.6mg/mL	1.96	0.6 μ g/mL

Figure 16

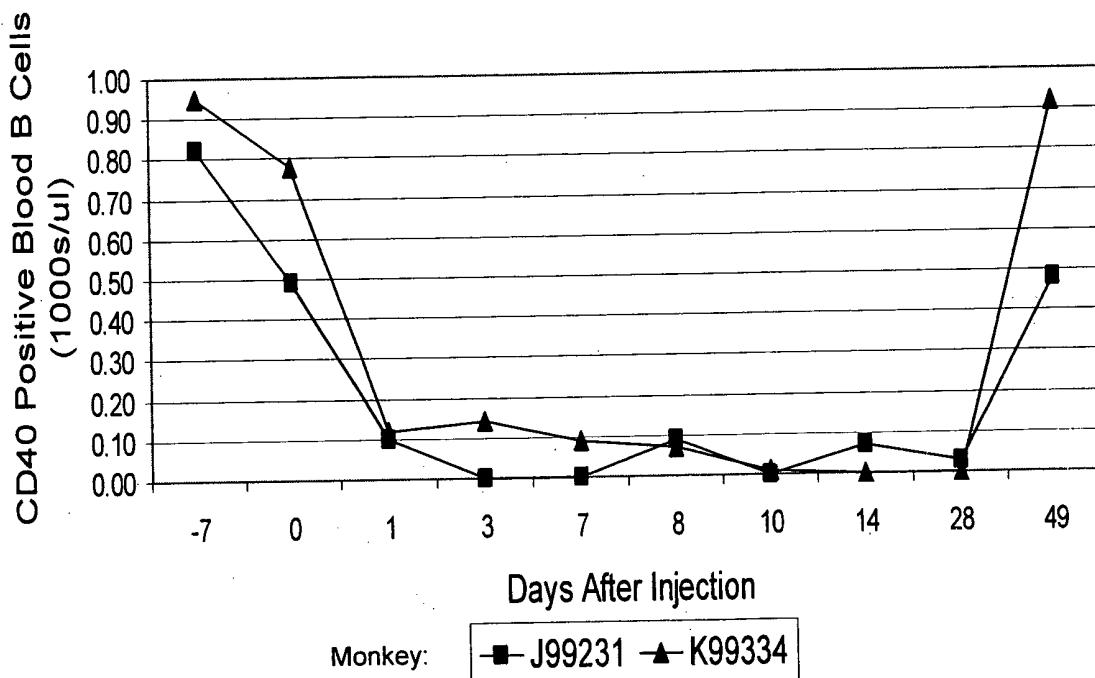
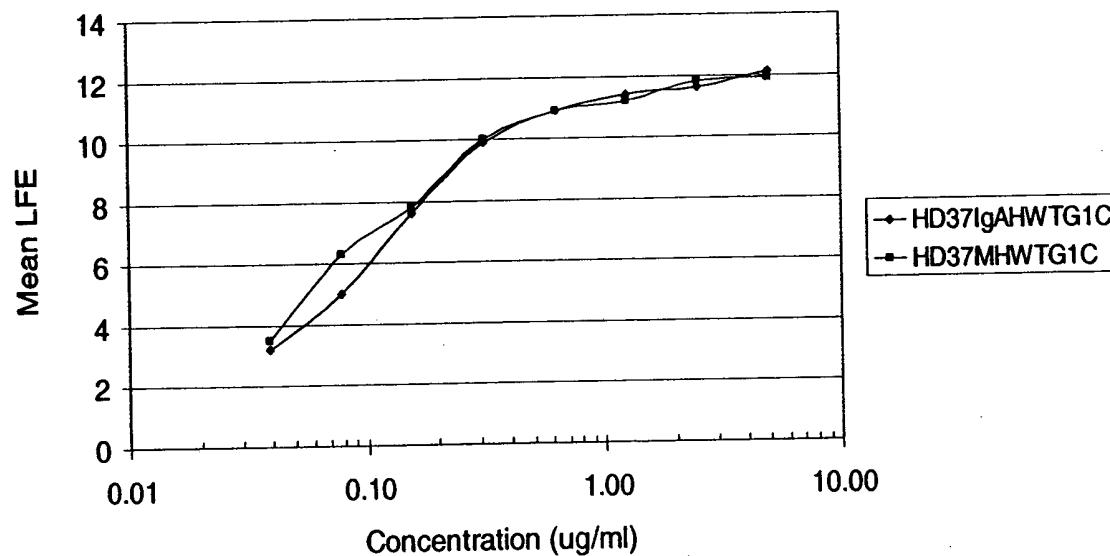


FIGURE 17

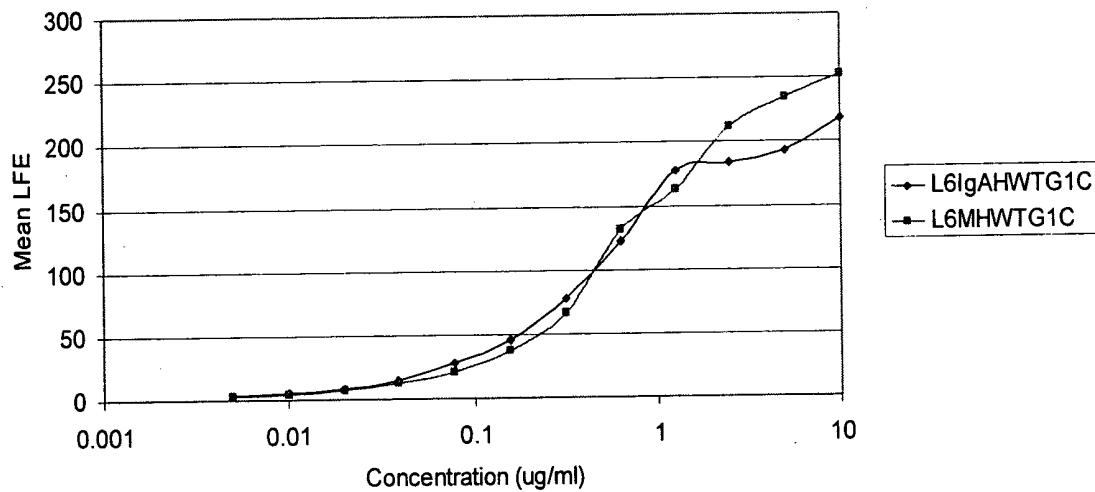
**Standard Curve of HD37 scFvIg Derivative
Binding to B Cells**



Clone/Isolate	Mean LFE at 1:100	Estimated Concentration
Bulk IgAHWTG1C	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
Bulk MHWTG1C	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

FIGURE 18

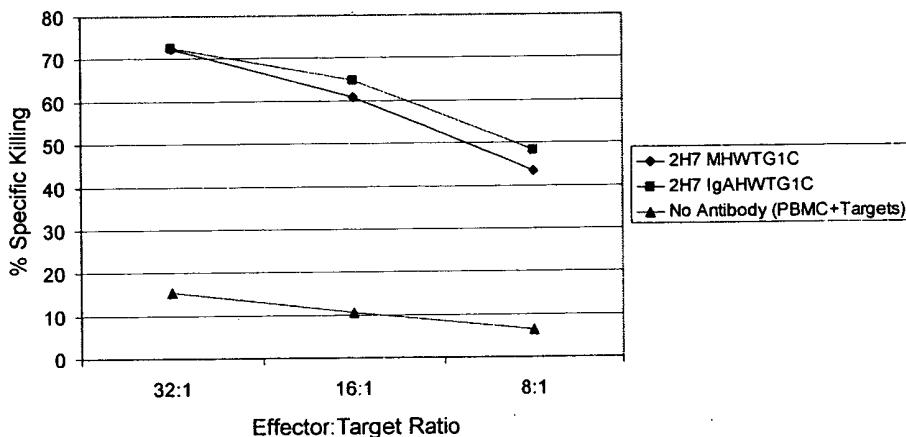
L6 scFvlg Standard Curves



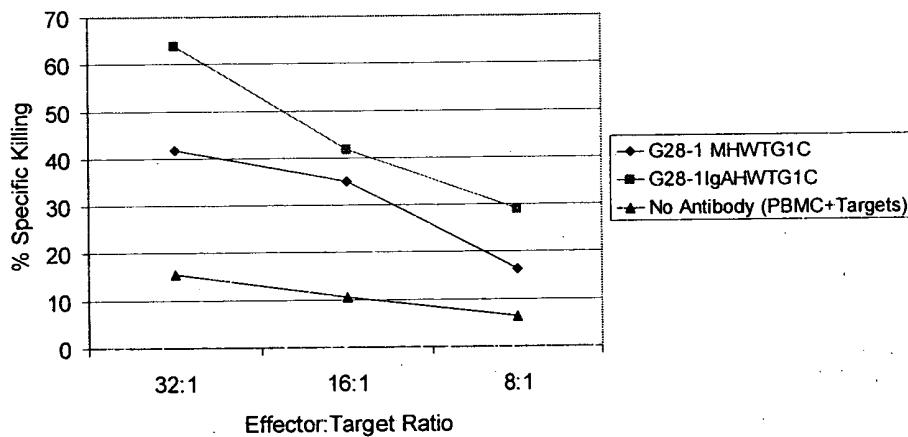
<u>Construct</u>	<u>Mean LFE 1:20</u>	<u>Estimated Concentration</u>
L6 IgAHWTG1C unamplified CHO sup	51.1	6.25 ug/ml
L6 IgGMHWTG1C unamplified CHO sup	23.0	3.2 ug/ml

FIGURE 19

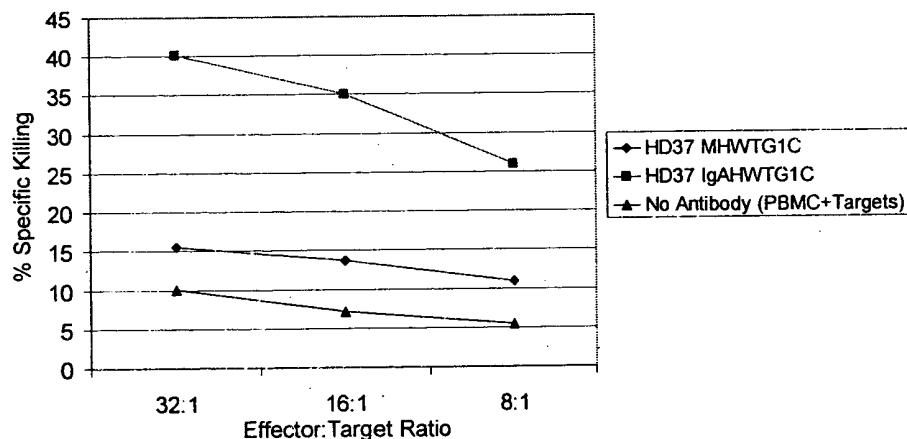
A. 2H7 (anti-CD20) scFv Derivatives



B. G28-1 (anti-CD37) scFv Derivatives

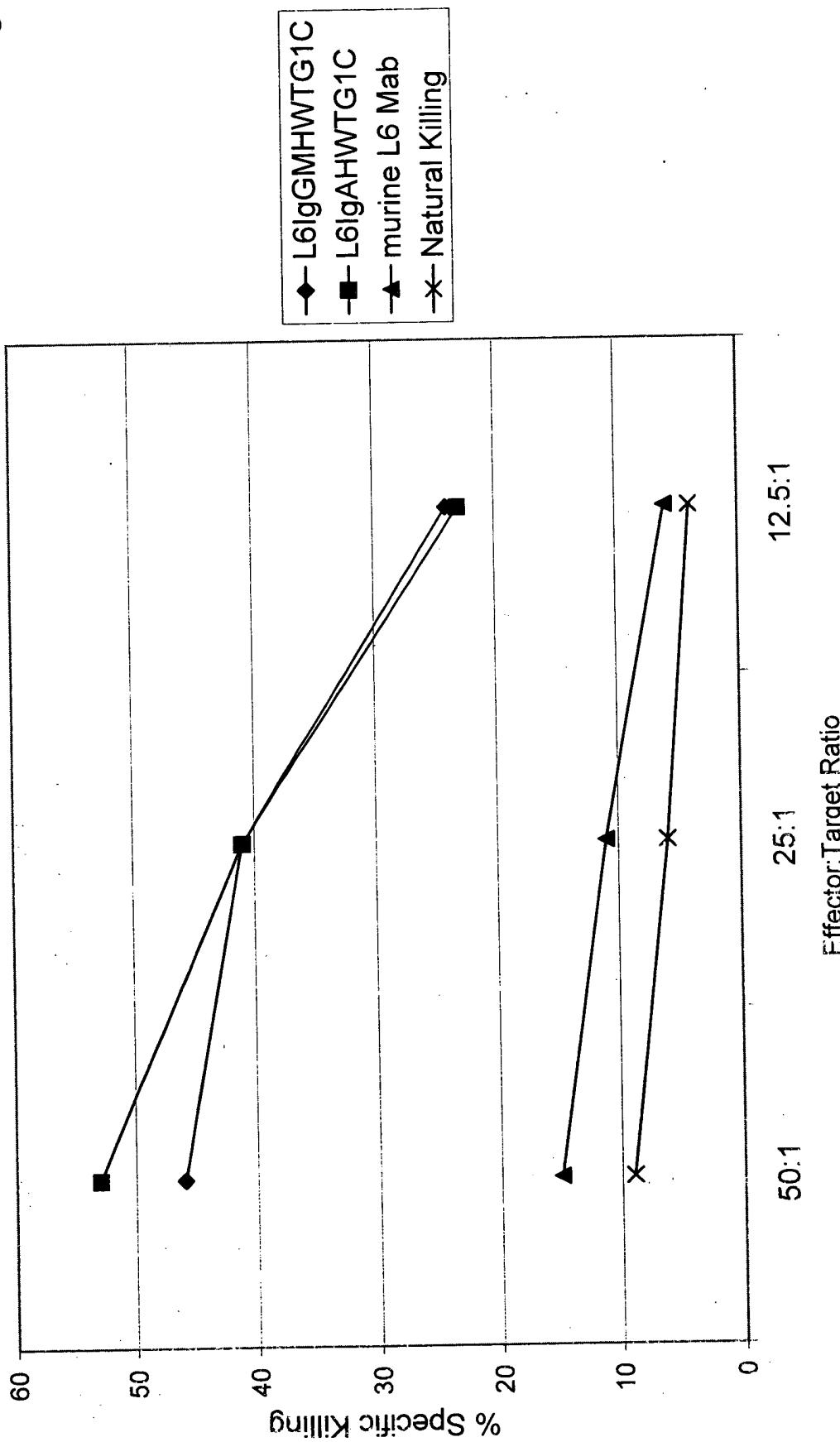


C. HD37 (anti-CD19) scFv Derivatives



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FIGURE 20



Effector:Target Ratio

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EXPRESS MAIL NO. EL 755733415US**

Figure 21

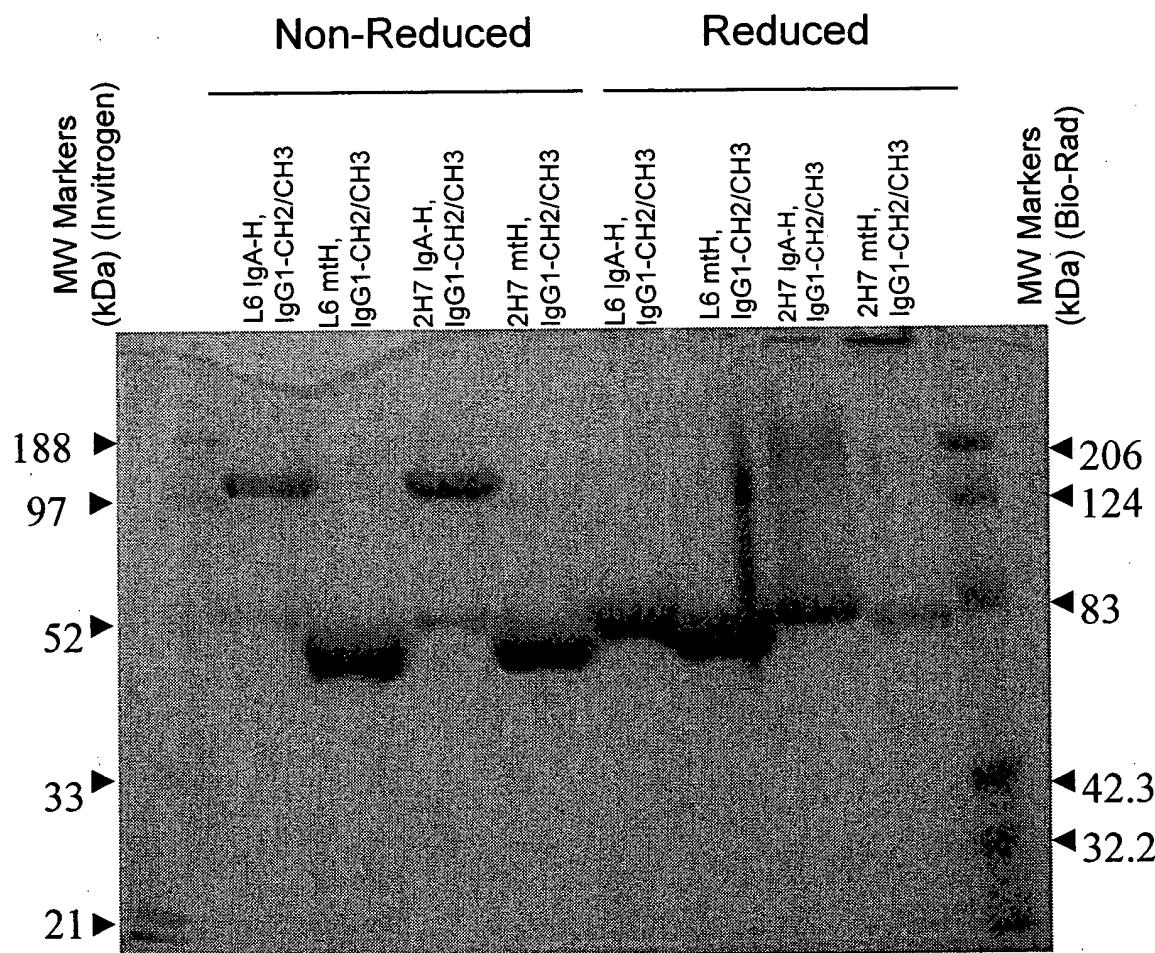


Figure 22

